JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2002, p. 306–308 Vol. 40, No. 1
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.1.306–308.2002

Diagnosis of Placental Malaria

Frank P. Mockenhaupt,[1]* Ulrike Ulmen,[1] Christiane von Gaertner,[2] George Bedu-Addo,[3] and Ulrich Bienzle[1]

Institute of Tropical Medicine, Charité, Humboldt University, Berlin, Germany,[1] and Presbyterian Mission Hospital, Agogo, Asante Akim,[2] and Komfo Anoyke Teaching Hospital, School of Medical Sciences, University of Science and Technology, Kumasi,[3] Ghana

Received 2 August 2001/Returned for modification 17 September 2001/Accepted 28 October 2001

In a group of 596 delivering Ghanaian women, the sensitivities of peripheral blood thick film microscopy, ICT Malaria P.f/P.v test, and PCR in detecting microscopically confirmed placental *Plasmodium falciparum* infection were 42, 80, and 97%, respectively. In addition to the gross underestimation of placental malaria by peripheral blood film microscopy, submicroscopic infections were found to be a risk factor for maternal anemia.

Placental infection with *Plasmodium falciparum*, i.e., sequestration of parasites in intervillous spaces, contributes to maternal morbidity, low birth weight, and preterm delivery (4, 7). Microscopy of peripheral blood films fails to identify a considerable proportion of these infections (3, 5). Therefore, postpartum microscopy of placental blood films has been used as a more accurate indicator (3). Yet, for both clinical and epidemiological reasons, sensitive methods are needed to assess placental malaria before delivery. Detection of the secretory malarial antigen histidine-rich protein 2 (HRP2) has recently been proven useful to diagnose placental *P. falciparum* infection in pregnant women (3). So far, the sensitivity of PCR assays in this regard is unknown. Moreover, the clinical relevance of submicroscopic *P. falciparum* infections in pregnancy is not well established (5). One aim of the present study was to determine the performance of PCR assays in the diagnosis of placental malaria in comparison to microscopy and to the detection of HRP2. Therefore, we assessed the prevalence of *P. falciparum* in placental and peripheral blood samples from 596 delivering women in Agogo, Ashanti Region, Ghana, by microscopy, detection of HRP2, and PCR assays. We then set placental blood parasitemia examined by the three methods as a reference and determined the sensitivity of each of the corresponding diagnostic methods for peripheral blood. In addition, we looked at the impact of submicroscopic *P. falciparum* infections on anemia, chosen as a crude marker of maternal morbidity. The study protocol was reviewed and approved by the Committee on Human Research Publication and Ethics, School of Medical Sciences, University of Science and Technology, Kumasi, Ghana, and informed consent was obtained from all participants.

For this study, women delivering at Agogo Hospital between January and August 2000 were recruited. Venous peripheral blood was collected into EDTA. Following expulsion and after a small incision had been made into the maternal surface of the placenta, blood from the intervillous space was collected with a syringe containing EDTA. Malaria parasites were counted microscopically on Giemsa-stained thick blood films per 100 high-power fields for placental samples and per 500 white blood cells for venous samples. HRP2 was detected by rapid immunochromatographic ICT Malaria P.f/P.v tests (Becton Dickinson, Heidelberg, Germany). Details of this assay are described elsewhere (1, 8). Briefly, 15 μl of blood was added to the test card pad containing colloidal gold-labeled antibodies. After addition of a lysis buffer, antigen-antibody complexes migrate up a test strip and are captured by immobilized antibodies. *P. falciparum* positivity was recorded if the HRP2 line and the control line were visibly colored. Nested *P. falciparum*-specific PCR assays were performed as described elsewhere (6). All examinations were blinded. Hemoglobin (Hb) concentrations were measured by a HemoCue photometer (Ångelholm, Sweden). Anemia was defined as an Hb level of <11 g/dl (9). Complete parasitological and hematological data were available for 596 women.

By microscopy, placental blood parasitemia occurred in 32% of the women. Parasite densities ranged from a mean of 0.01 to 1,600 per high-power field. By the ICT Malaria P.f/P.v test, placental blood infection was diagnosed in 38% of the women, and by PCR it was diagnosed in 56% of the women.

Setting microscopically confirmed placental parasitemia as a reference, the sensitivity of detecting *P. falciparum* in peripheral blood was lowest for microscopy (42%), about twice as high for the ICT Malaria P.f/P.v test (80%), and close to 100% for PCR. These differences in sensitivity were similar when the results of testing placental blood samples by ICT Malaria P.f/P.v or PCR were used as a reference (Table 1). The sensitivities of detecting microscopically confirmed placental parasitemia in peripheral blood increased with the placental parasite load for all three diagnostic means (Table 2). Resolved infections, i.e., no parasites discernible in placental blood films but macrophages containing malaria pigment, occurred in 7% (44 of 596) of the women. Of these, peripheral blood microscopy, ICT Malaria P.f/P.v test, and PCR were positive in 5, 32, and 57%, respectively.

Hb concentrations ranged between 4.6 and 16.4 g/dl (mean, 11.5 g/dl). We grouped peripheral blood *P. falciparum* infections into the following groups: (i) no infection, (ii) detectable by PCR only, (iii) detectable by ICT Malaria P.f/P.v test but not by microscopy, and (iv) microscopically confirmed parasitemia. Then, we analyzed the impact of these types of infec-

* Corresponding author. Mailing address: Institut für Tropenmedizin, Spandauer Damm 130, 14050 Berlin, Germany. Phone: 49 30 30116 750. Fax: 49 30 30116 888. E-mail: frank.mockenhaupt@charite.de.

TABLE 2. Sensitivities of detecting *P. falciparum* in peripheral blood according to placental parasite load

Placental parasite load (parasites/field) by microscopy[a]	No. of positive samples	Sensitivities (% [95% CI]) of peripheral blood tests[b]		
		Microscopy	ICT Malaria P.f/P.v test	*P. falciparum* PCR
<1	108	21 (16–26)	67 (59–74)	95 (90–98)
1–10	49	65 (54–74)	98 (89–100)	98 (88–100)
>10	31	77 (62–89)	100	100

[a] The mean number of parasites per high-power microscopy field is shown.
[b] 95% CI, 95% confidence interval.

tion on anemia (Table 3). Anemia occurred significantly more frequently in women with microscopically confirmed parasitemia or in those with a submicroscopic infection but a positive ICT Malaria P.f/P.v test than in uninfected individuals. Submicroscopic peripheral blood infection detectable exclusively by PCR was associated with lowered Hb concentrations but not with anemia per se (Table 3).

Microscopy of peripheral thick blood films fails to detect more than half of the *P. falciparum* infections diagnosed by microscopy of placental blood films. This may be because peripheral parasitemia is below the threshold of microscopy or parasites may be sequestered in placental tissue and evade circulation. Because *P. falciparum* secretes HRP2, this protein can be spotted by dipstick tests in blood of women with exclusive placental infection. Similar to findings from Cameroon (3), the probability of detecting HRP2 in blood correlated with the placental parasite load. In 181 Cameroonian women, the sensitivity of diagnosing microscopically confirmed placental parasitemia using the ICT Malaria P.f test in peripheral blood samples was 89% (3). The sensitivity of parasite PCR was not determined in that study.

Identification of placental malaria by peripheral blood PCR showed the highest sensitivity. This may result from very low peripheral parasitemia or from remaining DNA of recently phagocytosed parasites. However, in mice no DNA of *Plasmodium chabaudi* could be demonstrated two days after injection of dead parasites (2). Both low-level parasitemia and remnant DNA may account for the finding that in more than half of the women with resolved placental infections, i.e., presence of malaria pigment without parasites, peripheral blood PCR was positive.

Peripheral parasitemia below the threshold of microscopy constitutes a risk for anemia. PCR assays show the highest

TABLE 3. Impact on anemia of *P. falciparum* infections diagnosed by microscopy, ICT Malaria P.f/P.v test, and PCR

Peripheral blood *P. falciparum* infection group	No. of samples with indicated result	% Anemic	Hb (mean ± SD) (g/dl)
None	301	25	11.9 ± 1.7
PCR positive only	100	30	11.4 ± 1.4[a]
ICT Malaria P.f/P.v positive, microscopy negative	105	49[a]	10.7 ± 1.9[a]
Microscopy positive	90	40[a]	11.1 ± 1.7[a]

[a] Significant difference from noninfected women ($P < 0.05$ by χ^2 test or Student's t test).

TABLE 1. Sensitivity and specificity of placental *P. falciparum* detection in peripheral blood samples by microscopy, ICT malaria P.f/P.v test, and PCR

Test for placental parasitemia and result	No. (%) of patients with indicated result	Sensitivities and specificities of peripheral blood tests					
		Microscopy		ICT Malaria P.f/P.v test		PCR	
		No. of positive samples	Sensitivity (% [95% CI])[a]	No. of positive samples	Sensitivity (% [95% CI])	No. of positive samples	Sensitivity (% [95% CI])
Microscopy							
Negative	408 (68)	11		42		103	
Positive	188 (32)	79	42 (38–45)	151	80 (75–85)	182	97 (93–99)
ICT Malaria P.f/P.v test							
Negative	372 (62)	6		12		77	
Positive	224 (38)	84	38 (35–39)	181	81 (78–83)	208	93 (89–96)
PCR							
Negative	265 (44)	1		8		8	
Positive	331 (56)	89	27 (25–27)	185	56 (54–57)	227	84 (82–85)

Test for placental parasitemia and result	Specificity (% [95% CI])
Microscopy	
Negative	
Positive	97 (96–99)
ICT Malaria P.f/P.v test	
Negative	
Positive	98 (97–99)
PCR	
Negative	
Positive	100 (98–100)

Test for placental parasitemia and result	Specificity (% [95% CI])
Microscopy	
Negative	
Positive	90 (88–92)
ICT Malaria P.f/P.v test	
Negative	
Positive	97 (95–98)
PCR	
Negative	
Positive	97 (94–99)

Test for placental parasitemia and result	Specificity (% [95% CI])
Microscopy	
Negative	
Positive	97 (93–99)
ICT Malaria P.f/P.v test	
Negative	
Positive	93 (89–96)
PCR	
Negative	
Positive	84 (82–85)

Test for placental parasitemia and result	Specificity (% [95% CI])
Microscopy	
Negative	
Positive	75 (73–76)
ICT Malaria P.f/P.v test	
Negative	
Positive	79 (77–81)
PCR	
Negative	
Positive	97 (94–99)

[a] 95% CI, 95% confidence interval.

sensitivity in detecting placental parasitemia but the infections found in addition to those identified by the ICT Malaria P.f/P.v test seem to have only limited clinical importance as far as maternal anemia is concerned. The impact of submicroscopic infections on fetal outcome warrants further examination. One advantage of rapid immunochromatographic assays is the little amount of training required when compared to microscopy, but they are far too expensive for use in the public health systems of most countries where malaria is endemic. Nevertheless, both these devices and PCR assays may prove useful in epidemiological studies and for the evaluation of antimalarial interventions in pregnancy in areas of endemicity.

We thank the women who participated in this study.

This work was supported by Charité grant 2000-512 and by an "Infectious diseases" grant of MSD, Germany, to F.P.M. and is part of the doctoral thesis of U.U. Becton Dickinson donated ICT Malaria P.f/P.v tests.

REFERENCES

1. **Garcia, M., S. Kirimoama, D. Marlborough, J. Leafasia, and K. H. Rieckmann.** 1996. Immunochromatographic test for malaria diagnosis. Lancet **347:** 1549.
2. **Jarra, W., and G. Snounou.** 1998. Only viable parasites are detected by PCR following clearance of rodent malarial infections by drug treatment or immune responses. Infect. Immun. **66:**3783–3787.
3. **Leke, R. F., R. R. Djokam, R. Mbu, R. J. Leke, J. Fogako, R. Megnekou, S. Metenou, G. Sama, Y. Zhou, T. Cadigan, M. Parra, and D. W. Taylor.** 1999. Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria. J. Clin. Microbiol. **37:**2992–2996.
4. **Menendez, C., J. Ordi, M. R. Ismail, P. J. Ventura, J. J. Aponte, E. Kahigwa, F. Font, and P. L. Alonso.** 2000. The impact of placental malaria on gestational age and birth weight. J. Infect. Dis. **181:**1740–1745.
5. **Mockenhaupt, F. P., B. Rong, H. Till, T. A. Eggelte, S. Beck, C. Gyasi-Sarpong, W. N. Thompson, and U. Bienzle.** 2000. Submicroscopic *Plasmodium falciparum* infections in pregnancy in Ghana. Trop. Med. Int. Health **5:**167–173.
6. **Snounou, G., S. Viriyakosol, X. P. Zhu, W. Jarra, L. Pinheiro, V. E. do Rosario, S. Thaithong, and K. N. Brown.** 1993. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol. Biochem. Parasitol. **61:**315–320.
7. **Sullivan, A. D., T. Nyirenda, T. Cullinan, T. Taylor, S. D. Harlow, S. A. James, and S. R. Meshnik.** 1999. Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. J. Infect. Dis. **179:**1580–1583.
8. **Tjitra, E., S. Suprianto, M. Dyer, B. J. Currie, and N. M. Anstey.** 1999. Field evaluation of the ICT Malaria P.f/P.v immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with a presumptive clinical diagnosis of malaria in Eastern Indonesia. J. Clin. Microbiol. **37:**2412–2417.
9. **World Health Organization.** 1972. Nutritional anaemias. WHO Tech. Rep. Ser. 503.